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Research paper

Leaf-trait responses to irrigation of the endemic fog-oasis tree *Myrcianthes ferreyrae*: can a fog specialist benefit from regular watering?

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Myrcianthes ferreyrae is an endemic, endangered species, with a small number of individuals located only in hyperarid, fog-oases known as *lomas* along the Peruvian desert in southern Peru, where fog is the main source of water. Following centuries of severe deforestation, reforestation with this native species was conducted in the Atiquipa *lomas*, Arequipa-Perú. On five slopes, five 2-year-old seedlings were irrigated monthly with water trapped by raschel-mesh fog collectors, supplementing natural rainfall with 0, 20, 40, 60 and 80 mm month⁻¹ from February to August 2008. We measured plant growth, increment in basal diameter, height and five leaf traits: leaf mass area (LMA), leaf carbon isotope composition ($\delta^{13}\text{C}$), nitrogen per leaf area, total leaf carbon and stomatal density; which are indicative of the physiological changes resulting from increased water supply. Plant growth rates, estimated from the variation of either shoot basal diameter or maximum height, were highly correlated with total biomass. Only LMA and $\delta^{13}\text{C}$ were higher in irrigated than in control plants, but we found no further differences among irrigation treatments. This threshold response suggests an on–off strategy fitted to exploit pulses of fog water, which are always limited in magnitude in comparison with natural rain. The absence of a differential response to increased water supply is in agreement with the low phenotypic plasticity expected in plants from very stressful environments. Our results have practical implications for reforestation projects, since irrigating with 20 mm per month is sufficient to achieve the full growth capacity of this species.

Keywords: fog, leaf mass area, *lomas*, *Myrcianthes ferreyrae*, Peruvian desert, $\delta^{13}\text{C}$

Introduction

Lomas ecosystems are fog oases of the Peruvian and Atacama deserts that form an hyperarid belt close to the Pacific Ocean coast from northern Peru to northern Chile (7–28°S latitude) (Dillon et al. 2009). These deserts are considered to be among the driest on the planet (Rundel et al. 1991), where Cereceda et al. (2008), using 30 years of meteorological records, estimated that total yearly precipitation in the Atacama Desert amounted to 0.8 mm. As in other hyperarid areas (Henschel and Seely 2008), fog is the main water source at the *lomas*

(Cereceda et al. 1999). The vegetation, mainly trees, intercepts fog with its aerial structures, and throughfall and stemflow drive important water quantities into the soil (Dawson 1998, Ebner et al. 2011). This process has been simulated using fog collectors (Schemenauer et al. 1987), which have been set up and tested in these deserts (Schemenauer and Cereceda 1993, Larrain et al. 2002, Cereceda et al. 2008, Sotomayor and Jiménez 2008, Westbeld et al. 2009). The amount of water trapped by these collectors in other hyperarid environments reaches maxima of 1.0, 5.7 and 7.0 mm day⁻¹ for the

Namib Desert (Henschel and Seely 2008), the west coast of South Africa (Olivier 2002) and the Atacama Desert (Cereceda et al. 2008), respectively.

In the *lomas* ecosystems, reforestation has been conducted with the aim of restoring endemic and/or economic tree species, which are now less abundant due to old cutting practices (Suasnabar et al. 2004, Balaguer et al. 2011). Unfortunately, there is little information on reforestations using fog water (Estrela et al. 2009, Balaguer et al. 2011), and no studies assessing plant response to irrigation treatments in *lomas* ecosystems have been published. Our study species was *Myrcianthes ferreyrae* (McVaugh) McVaugh, an evergreen perennial tree. This fog specialist is an endemic species of *lomas* formations on the south coast of Peru (Kawasaki and Holst 2006), and it is currently only located in some patches of the Atiquipa and Taimara *lomas*, Arequipa, Peru (Suasnabar et al. 2004). *Myrcianthes ferreyrae* is listed in the official classification of endangered species of wild flora protected by Peruvian legislation (D.S. No. 043-2006-AG) as 'critically endangered' according to the red list criteria of the International Union for Conservation of Nature (IUCN 1999).

Leaf mass area (LMA), foliar isotope carbon composition ($\delta^{13}\text{C}$) and nitrogen concentration per leaf area (and other leaf traits) were measured in our study species with the aim of assessing the response of planted seedlings to different irrigation treatments based upon fog water. These leaf traits are related to plant growth and damage caused by herbivory (e.g. Antunez et al. 2001, Ruiz-Robledo and Villar 2005, Poorter and Garnier 2007, Poorter et al. 2009), which is considered to be the main causes of injury and mortality of reintroduced plants (Menges 2008, Godefroid et al. 2011), water use efficiency (Farquhar and Richards 1984) and carboxylation capacity (Hanba et al. 1999, Wright et al. 2001). The measured traits were used to assess plant response to water deficit (e.g., Gratani and Varone 2004, Haase et al. 1999, Ramírez et al. 2009), altitudinal gradients or changes in temperature (e.g., Vitousek et al. 1990, Suzuki 1998, Zhang et al. 2005, Mendez-Alonzo et al. 2008, Atkinson et al. 2011), and other stress factors. In previous afforestation and reforestation studies, leaf nitrogen concentration, LMA and $\delta^{13}\text{C}$ have been analysed to select the most adequate species based on a functional group basis (Delagrange et al. 2008), and to understand the effect of different practices on plant resource use efficiency (Querejeta et al. 2008). Our goals involved (i) assessing the degree of phenotypic plasticity of leaf traits and the growth response to a regular water supplementation by irrigation with fog water and (ii) inferring from the analysis of leaf functional traits how this species responds to regular watering. Due to the fact that water use by fog-specialist species relies on fog availability in high water deficit situations (Dawson 1998), we hypothesize a gradual improvement of the physiological status, indicated by leaf traits and growth, in

response to increasing water inputs. Our second hypothesis is that the leaf traits might reflect the interaction of our irrigation treatments with other environmental factors such as light intensity or damage due to herbivory.

Materials and methods

Study area

Our experiment was performed in the restoration area of the Atiquipa *lomas*, on the south coast of Peru ($15^{\circ}42'\text{S}$; $74^{\circ}18'\text{W}$) in the Peruvian desert, in Caraveli province, Arequipa (Figure 1). From August 2002 to December 2010 at the 'Barrera' Meteorological Station ($15^{\circ}45'\text{S}$; $74^{\circ}02'\text{W}$), the average annual rainfall in our study area was 66.3 mm, distributed into 83 and 17% in the 'wet-cool' (June–November) and the 'dry-hot' (December–May) season, respectively. The average monthly temperatures in the 'wet-cool' and 'dry-hot' seasons were 15.4 and 21.0 °C, respectively. The fog from the Pacific Ocean, occurring in the 'wet-cool' season, can provide an additional water input of 20.7 mm day⁻¹, the average value from the water harnessed by our fog collectors (see below) during 2002–2011. The soil has a sandy clay loam structure with 51.2, 26.8 and 22% of sand, silt and clay, respectively, 5.8% of organic matter, 15% of C/N, 5.3 pH and 0.62 mS cm⁻¹ of electric conductivity (Coaguila 2010).

Vegetation is mainly dominated by annual species growing in the 'wet-cool' season such as *Nicotiana paniculata*, *Alternanthera ferreyrae* and *Grindelia tarapacana*. The perennial shrub and tree species play an important functional role in the water input of this ecosystem because they serve as natural fog collectors. The most prominent woody perennial species in the Atiquipa *lomas* are *Caesalpinia spinosa*, *Duranta armata*, *Citharexylum flexuosum* and *Vasconsellea candicans* (Coaguila 2010). Approximately 500 adult individuals of *M. ferreyrae* were reported by our team in the Atiquipa *lomas* in 2006 (L. Villegas, personal communication).

Irrigation treatments with fog water and experimental design

In the Atiquipa *lomas*, 12 fog collectors were set up in the study area (<http://www.irecaunsa.com/unsapadova.html>), each of which is a low-cost 48 m² flat panel (12 m long × 4 m high) comprising a polypropylene raschel mesh (Schemenauer and Cereceda 1994) supported by two 5-m-high beams. A gutter, located at the lower part of the flat panel, channels the water to a pipe that transports it to four water reservoirs with capacities of 60, 300, 600 and 1000 m³, respectively. Another system of pipes transports the water from the reservoirs to five slopes containing a plantation of 2-year-old specimens of *M. ferreyrae*. Four irrigation treatments (20, 40, 60, 80 mm month⁻¹) and one control (no watering) were established randomly with five seedlings per treatment on each of the five aforementioned

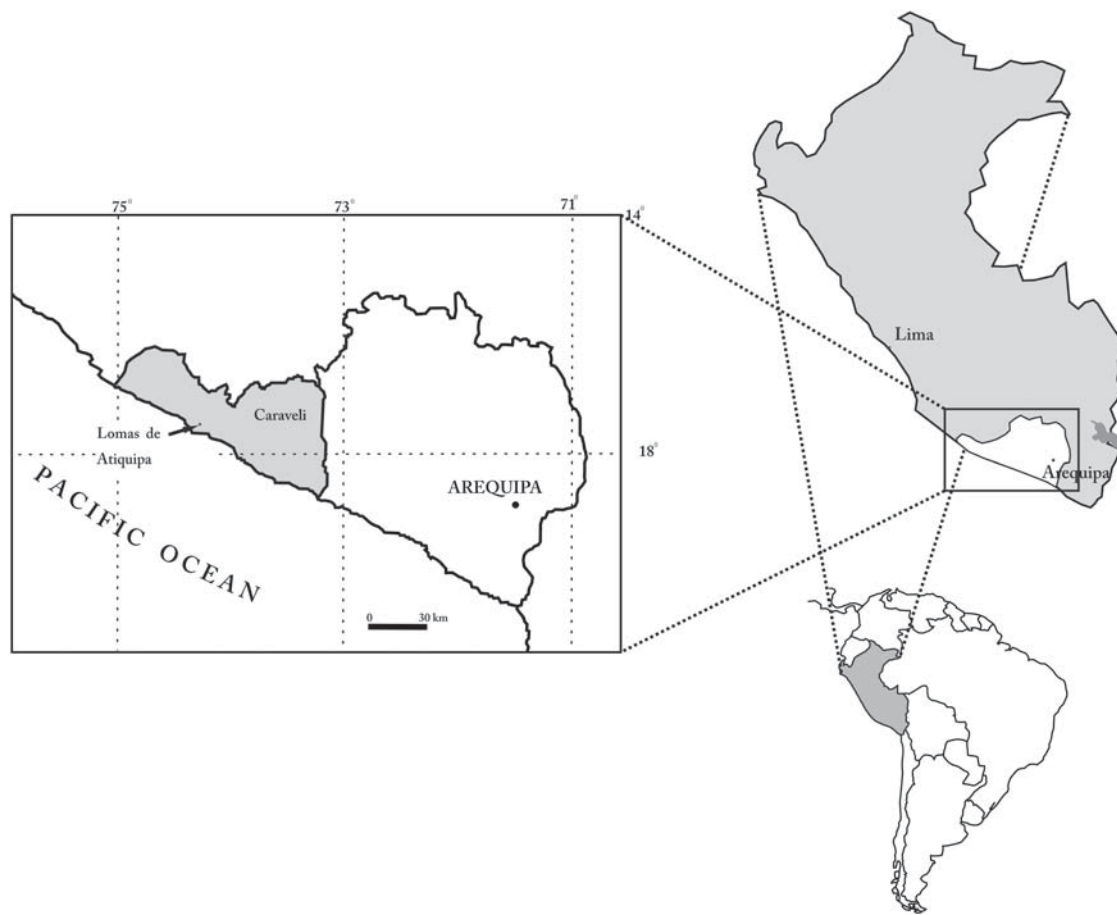


Figure 1. Study area map.

slopes. Watering was performed manually at the beginning of the assessed months from February to August 2008.

Growth assessment

The maximum height and basal stem diameter of each seedling were measured at the beginning and the end of the study period. Growth was assessed, from the percentage of variation from the first measurement (Δ), as

$$\Delta X = [(X_{\text{August}} - X_{\text{February}}) / X_{\text{February}}] \times 100 \quad (1)$$

where X is seedling maximum height or basal stem diameter. Twelve different-sized seedlings were removed, dried and weighed, and above and below biomass was separated in order to fit calibration functions among seedling maximum height and stem basal diameter vs. dry total biomass (aboveground + below-ground biomass). The herbivory damage caused by insect larvae was estimated as the percentage of damaged leaves in relation to the total number of leaves on a labelled branch.

Leaf traits

In August 2008, we collected four fully expanded young leaves of a similar size and belonging to the same cohort

located in the middle of the each seedling. In the laboratory, the leaves were scanned and leaf area (A) was measured using ImageJ software (1.41o version, National Institutes of Health, Bethesda, MD, USA). The leaves were re-hydrated for 2 days in deionized water at 10 °C. Then we estimated leaf volume (V) following the same procedure of Mitchell et al. (2008) based on the Archimedes principle, which involves measuring the weight gain (on a weighing scale with 0.01 mg of accuracy) of a beaker with water of a known mass following complete immersion of the re-hydrated leaf. Following Poorter et al. (2009), we calculated leaf volume per area ($LVA = V/A$) as a surrogate of leaf thickness. The epidermis of the leaf abaxial side was impressed in a dental resin (3M ESPE Dental Products, St. Paul, MN, USA) in order to measure stomatal density (SD) following Geisler et al.'s (2000) protocol. A layer of nail polish was poured on the impressed leaf size, carefully removed after drying, and then placed on a slide cover and analysed in an inverted microscope (Leica DM IRB model, Leica Microsystems GmbH, Wetzlar, Germany). It was then connected to a digital camera system (Leica DC 300F model, Leica Microsystems GmbH) and the stomatal number was assessed in four images (located in the centre of the leaf) in a 100× field (0.58 mm²) per leaf.

To estimate foliar dry mass (DM), the leaves were dried for 2 days at 70 °C and weighed. Leaf mass area (LMA) and leaf density (LD) were calculated as

$$\text{LMA} = \text{DM} / A \quad (2)$$

$$\text{LD} = \text{DM} / V \quad (3)$$

The dry leaves were ground with a mill ball and sent to the Stable Isotope Facility of Madrid's Autónoma University, where the leaf carbon isotope composition ($\delta^{13}\text{C}$) was analysed as

$$\delta^{13}\text{C}(\text{‰}) = [(R_{\text{Sample}} / R_{\text{Standard}})] \times 1000 \quad (4)$$

where R is $^{13}\text{C}/^{12}\text{C}$ proportion of the leaf sample and the Pee Dee Belemnite (as standard) and was determined by continuous flow isotope ratio mass spectrometry (Europe Scientific Hydra 20/20, Crewe, Cheshire, UK). Foliar N and C were determined with an automated 1500 Carbo Erba elemental analyser (EM-I, Stanford, CA, USA). Nitrogen content per unit area (N_{area}) was calculated as total foliar N divided by A . $\delta^{13}\text{C}$, foliar C and N_{area} were measured from four leaves collected per individual in a total of 75 seedlings (3 individuals \times 5 treatments \times 5 slopes).

Statistical analyses

The measured variables were analysed by means of one-way randomized block analysis of variance (ANOVA), using irrigation treatments and slopes as blocks. Linear regression analyses were performed between LVA vs. LMA and DM vs. LMA. The association among LMA, $\delta^{13}\text{C}$, SD, leaf carbon concentration and N_{area} was analysed by means of principal components analysis (PCA). We conducted correlation analyses (Spearman correlation index) to assess the relationship between the variables and the extracted principal components. We used SPSS 17.0 version software for Windows (SPSS, Chicago, IL, USA) to run the aforementioned analysis.

Table 1. F values of one-way randomized block ANOVA testing irrigation treatments (control, 20, 40, 60, 80 mm month $^{-1}$) in five blocks (slopes) in: variation of the stem basal diameter and seedling maximum height from the first assessment (ΔDiam and ΔHeight , respectively); leaf damage; LMA, leaf mass area; $\delta^{13}\text{C}$, leaf carbon isotope composition; SD, stomatal density; %C, leaf carbon concentration; N_{area} , nitrogen content per unit area. ** $P < 0.01$, * $P < 0.05$, n.s. $P > 0.05$.

	Irrigation treatments	Blocks
ΔDiam	6.6**	1.4 n.s.
ΔHeight	3.2*	1.4 n.s.
Leaf damage	0.5 n.s.	1.5 n.s.
LMA	5.0**	3.5**
$\delta^{13}\text{C}$	2.5*	0.4 n.s.
SD	6.3**	6.4**
%C	2.6**	8.6*
N_{area}	2.3 n.s.	0.4 n.s.

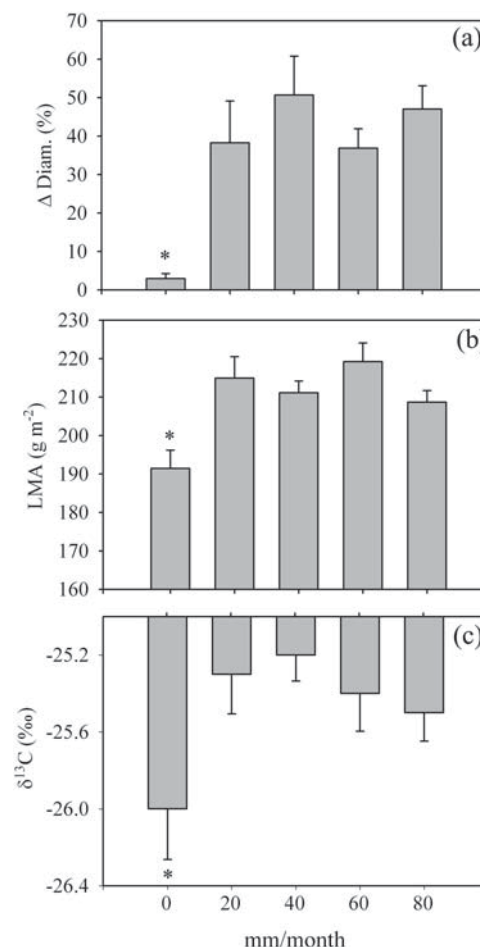


Figure 2. Leaf traits and plant growth response to the irrigation treatments: (a) variation in basal diameter from the first assessment (ΔDiam), (b) LMA and (c) $\delta^{13}\text{C}$. *Indicates significant differences (at $P < 0.05$) detected by the post hoc Tukey test of the ANOVA. Error bars represent SE.

Results

Growth response

Stem basal diameter showed a stronger relationship with aboveground + belowground biomass ($y = 7.5x - 37.8$; $r^2 = 0.95$; $P < 0.01$) than with seedling maximum height ($y = 1.3x - 24.7$; $r^2 = 0.76$; $P < 0.05$). At the study onset (February 2008), no significant difference was detected in the basal diameter between control seedlings and those submitted to the four irrigation treatments ($F = 1.21$, $P = 0.27$), which indicates that the study seedlings were at a similar growth. Significant differences among watering treatments were detected in the variation in stem basal diameter (ΔDiam) and in seedling maximum height (ΔHeight) in relation to the first assessment (Table 1). Plants in the control treatment showed significantly lower values of ΔDiam and ΔHeight than those submitted to the other watering treatments (Figure 2 and Table 2). The block effect was not significant in ΔDiam and ΔHeight (Table 1). The global average of leaf damage due to herbivory

Table 2. Mean (\pm SE) value of the variation in seedling height from the first assessment (Δ Height), leaf damage, SD, %C and N_{area} grouped for each irrigation treatment. Different letters indicate significant differences detected by the post hoc Tukey test of the one-way ANOVA (see Table 1) at $P < 0.05$.

	Treatments (mm month^{-1})				
	Control	20	40	60	80
Δ Height	$5.2 \pm 2.3a$	$12.5 \pm 2.0b$	$14.6 \pm 3.0b$	$15.0 \pm 3.0b$	$19.7 \pm 3.0b$
Leaf damage (%)	$14.6 \pm 3.3a$	$14.5 \pm 2.6a$	$10.9 \pm 3.1a$	$13.6 \pm 2.9a$	$15.0 \pm 2.7a$
SD (stomata mm^{-2})	$260.6 \pm 8.3a$	$260.3 \pm 5.9a$	$265.7 \pm 6.7a$	$271.1 \pm 7.8a$	$238.0 \pm 4.4b$
%C	$45.8 \pm 0.8a$	$46.9 \pm 0.4ab$	$46.5 \pm 0.5ab$	$46.9 \pm 0.4ab$	$47.5 \pm 0.5b$
N_{area} (g N m^{-2})	$2.9 \pm 0.2a$	$3.8 \pm 0.3a$	$3.2 \pm 0.2a$	$3.3 \pm 0.2a$	$3.7 \pm 0.2a$

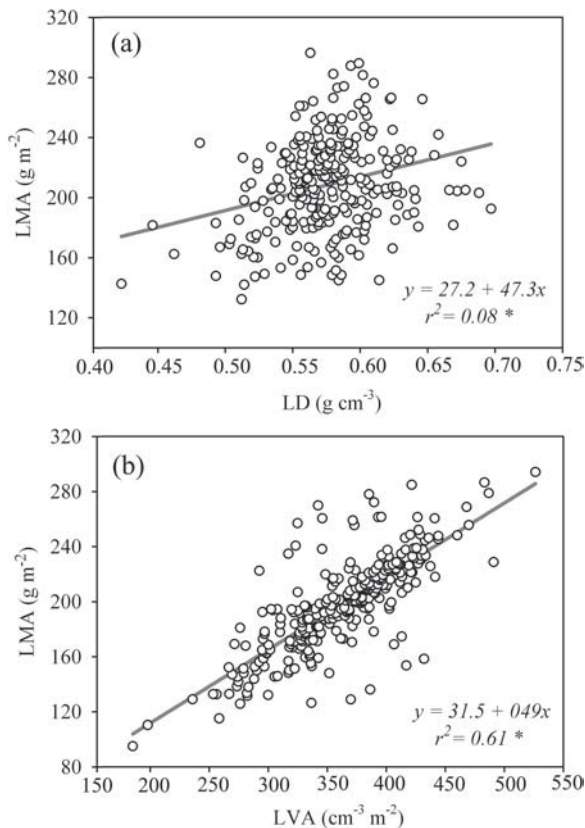


Figure 3. Relationship between LMA and its components: LD and LVA ~ thickness.

was 13.7 ± 1.3 %; this variable did not show significant differences among irrigation treatments or blocks (Tables 1 and 2).

Leaf-trait responses to irrigation treatments

The LMA (ranging from 105.7 to 352.3 g m^{-2}) and $\delta^{13}\text{C}$ (ranging from -27.0 to -23.7 ‰) were lower in the control than in the other irrigation treatments (Table 1 and Figure 2). Leaf mass area was explained by LVA ($r^2 = 0.61$), while no relationship was found between the former and leaf density ($r^2 = 0.08$) (Figure 3). The ANOVA analysis also detected significant differences among irrigation treatments in SD and leaf carbon concentration (%C) (see Tables 1 and 2). Thus,

Table 3. Spearman correlation index among the extracted components using multivariate PCA and foliar variables. **Mean significant values at $P < 0.01$ and $P < 0.05$, respectively. Values shown in italics are the main correlations with $r_{\text{Spearman}} > 0.6$.

	Component 1	Component 2	Component 3
SD	0.26	-0.71^{**}	0.35^{**}
$\delta^{13}\text{C}$	-0.77^{**}	-0.30^*	0.30^*
%C	0.41^{**}	0.30^*	0.80^{**}
N_{area}	-0.51^{**}	0.67^{**}	0.12
LMA	-0.70^{**}	-0.30^*	0.16
Eigenvalue	1.70	1.23	0.90
Cumulative variance (%)	34.1	58.7	76.7

SD (ranging from 198.8 to 360.4 stomata mm^{-2}) in the 80-mm-month $^{-1}$ treatment was lower than in the other irrigation treatments, while %C (ranging from 40.5 to 53.4%) was only different between the control and the maximum irrigation treatment (Table 2). No significant differences were found in the nitrogen content per unit area (N_{area} , ranging from 0.20 to 0.50 g N m^{-2}) or in the irrigation factor (Tables 1 and 2). Differences between blocks were not significant only in $\delta^{13}\text{C}$ and N_{area} , which reflects the spatial variability at the restoration site (Table 1).

Relationship among leaf traits

The three components extracted by PCA explained 76.7% of total variance (Table 3). Component 1 was highly negatively correlated with LMA and $\delta^{13}\text{C}$ ($r_{\text{Spearman}} = -0.77$ and -0.70 , respectively), while SD and N_{area} were negatively and positively correlated with component 2 ($r_{\text{Spearman}} = -0.71$ and 0.67 , respectively). Component 3 was highly positively correlated with %C ($r_{\text{Spearman}} = 0.80$) (Table 3). A high proximity and/or association between LMA and $\delta^{13}\text{C}$ was found in the multivariate space established by the three extracted components (Figure 4). The averages of the control (no watering) in LMA vs. N_{area} and $\delta^{13}\text{C}$ vs. N_{area} plots were far from the averages pertaining to the other irrigation treatments (Figure 5).

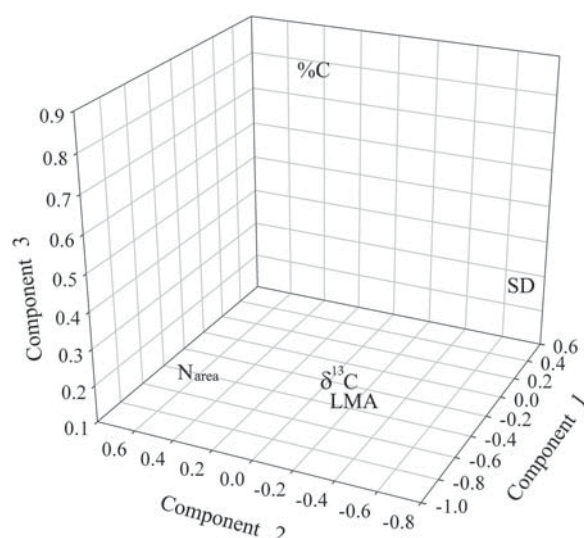


Figure 4. Ordination of the leaf variables in a 3D space from the three principal components extracted in the PCA. For specification of PCA and abbreviation of the variables, see Table 3.

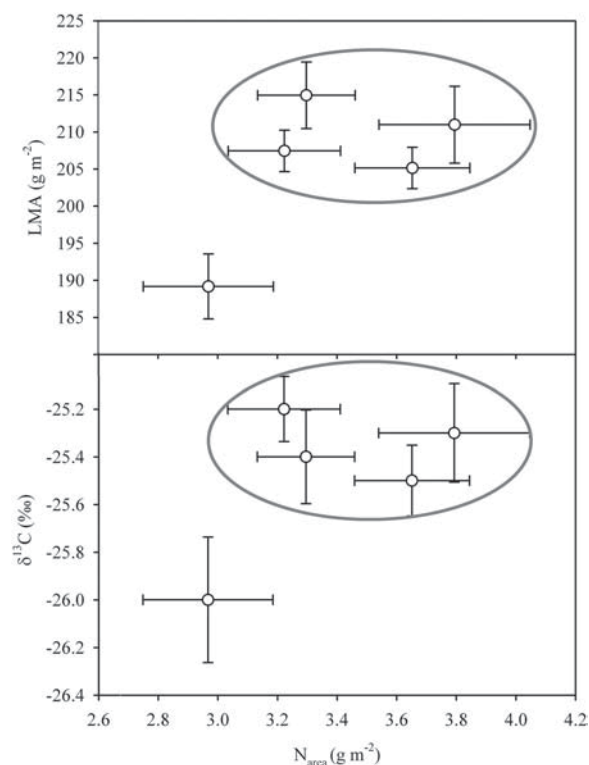


Figure 5. Relationship between LMA and $\delta^{13}\text{C}$ vs. N_{area} averaged for control plants and individuals subject to each of the irrigation treatments tested (circled values). Error bars represent SE.

Discussion

The seedlings of *M. ferreyrae* showed a significant increase in growth, LMA and $\delta^{13}\text{C}$ with water input (Figure 2). However, in disagreement with our initial hypothesis, this response was not gradual but was rather a threshold-type one. Our results

suggest that water use in our fog-specialist species might be limited by a low phenotypic plasticity, which is expected in specialized plants from poor or less favourable environments (Lortie and Aarssen 1996). In arid and semiarid areas, this specialized strategy of water uptake is more common than the opportunistic one, in which plants acquire water whenever and wherever it is available (Schwinning and Ehleringer 2001).

Growth response to irrigation

The different irrigation treatments tested in this research attempted to simulate water gains from fog intercepted by trees and reaching the understorey and soil via stemflow and through-fall. For example, in our study area, 96 mm day⁻¹ was collected as the maximum below a *C. spinosa* tree (L. Villegas, A. Ortega, C. Talavera and P. Jiménez, unpublished data). This value is close to those reported by Schemenauer and Cereceda (1994) below an *Olea europaea* tree (70 mm day⁻¹) in Masroob, Oman. Bigger trees with a large amount of leaves and branches are able to intercept higher fog volumes and perhaps enable higher water inputs to the vegetation below. Thus, for example, in redwood forests in California, fog water dripped from the vegetation in a range of 22–46 mm year⁻¹, corresponding to 13–45% of its annual transpiration (Dawson 1998). As seedlings are incapable of intercepting large amounts of fog water, our irrigation treatments attempted to simulate situations presenting different tree covers with no shading effect. The lack of proportional growth in response to the water increments tested by each watering treatment (Figure 2a) might be reflecting a threshold (20 mm month⁻¹) beyond which water increments do not cause a significant increase in growth. We hypothesize two implications of the growth asymptotic response to water input in the restoration of this species: (i) this threshold value (or values coming close) could be crucial in the regeneration phase of this species, representing the minimum amount of water required to improve the regeneration niche in these habitats, which is considered to constitute the bottleneck for successful species establishment (Poorter 2007); (ii) this threshold could provide a certain degree of reliability and trigger the growth of individuals belonging to the 'seedling bank', in which the aforementioned slow-growth seedlings remain in wait until ideal conditions promote their definitive establishment (Antos et al. 2005). However, *M. ferreyrae* shows a high LMA value (global average 210 g m⁻²) similar to some of the most sclerophyllous Australian shrubs (66–313 g m⁻², Niinemets et al. 2009). Because a high LMA is characteristic of slow-growth species (Wright et al. 2004, Poorter and Garnier 2007), a longer time of assessment might have been needed to detect growth differences among our watering treatments. Moreover, root/shoot ratio is usually higher in dryland species (Larcher 2003), and we therefore cannot rule out that our different irrigation treatments may have had a differential effect on belowground biomass. Additionally, carbon storage plays a key role in the response of

woody seedlings to drought (Piper 2011). We cannot discard differential carbon allocation to storage to the detriment of plant growth, which could help to withstand future water stress periods. More studies are required to understand the non-structural carbohydrates and root growth dynamics and their response to water inputs in the species of these fog-oasis ecosystems.

Leaf mass area components and response to irrigation treatments

Leaf volume per area and LD are components of LMA whose contributions become more important in different environmental situations (Lambers et al. 2008). Leaf density contribution is differentially relevant in stressful environments whereas LVA has more weight in environments with higher irradiance (Poorter et al. 2009). In our study, LVA explained 61% of LMA variability (Figure 3), which suggests that leaves belonging to individuals submitted to irrigation treatments might have been more exposed to solar radiation, possibly due to more horizontal leaf angles. Thus, N_{area} has been related to micro-environments with high irradiance with regard to optimizing canopy photosynthesis (Hirose and Werger 1987). In our study, individuals submitted to the watering treatments tended to exhibit higher values of this variable (with no statistical significance) than the control plants (Table 2, Figure 5). Under total sun exposure, a larger parenchyma develops (Onoda et al. 2008), resulting in a thicker mesophyll that may, in turn, enable more efficient use of incident light, as well as a higher capacity to safely dissipate excess photon energy.

The rise in structural carbohydrates (SC) is highly correlated with LMA increments driven by LD (Poorter et al. 2009). This SC synthesis is triggered in turn by damage resulting from herbivory (Schulze et al. 2002, Lambers et al. 2008). We found no significant differences in LD (data not shown) and leaf damage caused by herbivory (Table 2) between the control and the irrigation treatments. This result suggests that all the seedlings could have been subjected to a similar stimulation for SC synthesis, and a non-significant variation in LD among treatments can therefore be expected. Our measured leaf traits and the components thereof support the hypothesis that light-processing efficiency, driven by a thicker parenchyma, is more important than structural component synthesis in situations of higher water availability. This is in agreement with Lusk et al. (2008), who suggested that, in conditions of high light availability, the symplast per area fraction is greater than the structural fraction in leaves of evergreen shade-tolerant species.

Possible functional mechanisms driven by irrigation with fog water

A positive correlation between LMA and $\delta^{13}\text{C}$ has been found in inter-specific studies (Lamont et al. 2002, Hoffmann et al. 2005, Paula and Pausas 2006), in which comparisons were made with contrasted leaf types. The high association of LMA and $\delta^{13}\text{C}$, which were located closely in the multivariate 3D

space (Figure 4 and Table 3), and their correspondence with plant growth in our study species (Figure 2) highlight the coherence in leaf structure and photosynthetic (CO_2 diffusion and carboxylation) responses to water levels in fog-oasis arid environments. As in thick-leaved species, with high LMA like our study species, a significant decoupling exists between $\delta^{13}\text{C}$ and water use efficiency (WUE) (Marshall et al. 2007), it cannot be stated that WUE is increased by our watering treatments (Figure 2c). LMA affects mesophyll conductance (g_m) and consequently chloroplastic carbon and $\delta^{13}\text{C}$ (Seibt et al. 2008). A low g_m value resulting from a thicker mesophyll (Vitousek et al. 1990, Niinemets et al. 2009, but see Lauteri et al. 1997, Hanba et al. 1999) could cause an increase in chloroplastic carbon demand and, subsequently, in $\delta^{13}\text{C}$ (Lamont et al. 2002). Thus, N_{area} has been related to a higher Rubisco content (Hanba et al. 1999) and carboxylation (Wright et al. 2001). In our study, we found that watered plants tended to show a higher N_{area} and $\delta^{13}\text{C}$ than control plants (Figure 5b). We hypothesize that in the former, more intense Rubisco activity might reduce the discrimination against the heavy carbon isotope and the increased $\delta^{13}\text{C}$.

Our results are consistent with the reported drop in SD in response to water availability (Yang et al. 2004, Zhang et al. 2006), unlike what has previously been reported (Xu and Zhou 2008, Xu et al. 2009). Furthermore, SD has been positively related to WUE (Yang et al. 2004, Xu and Zhou 2008). The lack of a correlation between SD and $\delta^{13}\text{C}$, which is considered a useful variable for explaining stomatal formation (Nobuhito and Katsuya 2008), and the other assessed leaf traits prevent us from associating this variable with processes involved in gas exchange and carboxylation and, consequently, with WUE. We believe that the assessment of leaf gas exchange in our study species could be of great use in the future for two main reasons: (i) an uncoupling exists between $\delta^{13}\text{C}$ and WUE, particularly in sclerophyll species (Marshall et al. 2007, Seibt et al. 2008), and (ii) little is as yet known of the relationship between SD and gas exchange in plants (Xu and Zhou 2008).

Conclusions

Myrcianthes ferreyrae, an endemic, highly endangered specialist species of fog-oasis ecosystems, shows an improvement in growth and morpho-functional traits in response to water pulses from fog water. However, this response is not proportional to water input, which is possibly caused by the low phenotypic plasticity typical of species in highly stressed environments. This conclusion has very important practical consequences because a moderate water amount (20 mm month^{-1}) collected from fog is sufficient to irrigate 2-year-old seedlings of *M. ferreyrae*. Our results imply large water savings, which can be used for reforestation with other tree species. Due to the consistent response of LMA and $\delta^{13}\text{C}$

with plant growth to irrigation with fog water, our study highlights the use of these variables in assessing reforestation with endemic species (in our study, *M. ferreyrae*) in the *lomas* ecosystems. We hypothesize that increased irrigation to seedlings drives more irradiated leaves (possibly by more horizontal leaf angles) with a thicker mesophyll and a higher content of nitrogen per area; all these factors could enable a larger amount of photons to be processed and a more intense Rubisco activity, with the subsequent increase in plant growth. All these aspects, however, require specific experiments to help us understand the functional processes driven by water input by fog, a vital source for the maintenance of these fog oases in the Peruvian and Atacama deserts.

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